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**AQUEOUS PHARMACEUTICAL COMPOSITIONS OF 2,6-DIISOPROPYLPHENOL (PROPOFOL) AND THEIR USES**

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**AQUEOUS PHARMACEUTICAL COMPOSITIONS OF  
2,6-DIISOPROPYLPHENOL (PROPOFOL) AND THEIR USES**

10      1. CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit under 35 U.S.C. 119(e) to prior U.S. provisional Patent application Serial Nos. 60/399,490 filed July 29, 2002, 60/422,195 filed 10/29/2002, 60/436,979 filed January 30, 2002, 60/443,490 filed January 29, 2003; 60/462,450 filed on April 11, 2003; 60/464,314 filed on April 21, 2003; 60/470,403 filed on May 14, 2003; and 15 60/485,354 filed on July 7, 2003. The present application is also a continuation-in-part of U.S. patent application Serial Nos. 10/629,308 filed on July 29, 2003 and 10/677,747 filed on October 2, 2003, respectively. The present application also claims the benefit of related International Applications PCT/US03/23512 filed 7/29/2003 and PCT/US03/31086 filed October 2, 2003. Each of these prior applications is incorporated herein by reference, and it 20 its entirety.

2. FIELD OF THE INVENTION

The present invention relates to aqueous pharmaceutical compositions containing a lipophilic therapeutic agent. In particular, the invention provides aqueous pharmaceutical 25 compositions containing the compound 2,6-diisopropylphenol (propofol).

3. BACKGROUND OF THE INVENTION

The compound 2,6-diisopropylphenol is a well known anesthetic agent that is commonly known as propofol. The onset of anesthesia is largely controlled by a drug's 30 diffusion rate through the blood-brain barrier. Propofol is lipophilic, and this property helps that compound to provide rapid anesthetic action. However, the lipophilicity of propofol also renders that compound, which is a liquid at room temperature, relatively insoluble in water. As a result, propofol is commonly administered directly into the bloodstream (either by infusion or by bolus injection) as an oil-in-water emulsion. Such formulations typically

contain a lipid component to solubilize the drug. Lipids, however, are good substrates for bacterial growth and can also be incompatible with preservatives that are at least somewhat water soluble such as benzyl alcohol. Further, parenteral administration of large volumes of lipid emulsions and/or the administration of lipid emulsions over prolonged periods of time  
5 may result in hyperlipidemia.

Despite these shortcomings of such oil-in-water emulsions, propofol has been a successful anesthetic and is commercially available for human administration as Diprivan® Injectable Emulsion (AstraZeneca; Diprivan® is a trademark of Imperial Chemical Industries PLC). Propofol is also marketed for veterinary use as Rapinovet™ Anesthetic Injection  
10 (Schering-Plough Animal Health Corp.; Rapinovet™ is a trademark of Schering-Plough Veterinary Corp.) and as PropoFlo™ Anesthetic Injection (Abbott Laboratories; PropoFlo™ is a trademark of Abbott Laboratories).

Diprivan® Injectable Emulsion is a white, oil-in-water emulsion containing, in addition to 10 milligrams propofol per milliliter of emulsion, 100 mg soybean oil per mL,  
15 22.5 mg glycerol per mL, 12 mg egg lecithin per mL, 0.005% disodium edetate, and sodium hydroxide. Diprivan® Injectable Emulsion is indicated as a single-use parenteral product.  
Diprivan® contains disodium edetate to retard the growth of microorganisms in the event of  
accidental extrinsic contamination. However, Diprivan® can still support the growth of  
microorganisms. As acknowledged in the product insert, there have been reports in which  
20 failure to use antiseptic techniques when handling the emulsion was associated with  
microbial contamination and associated medical complications. It is therefore necessary to  
discard tubing and unused portions of Diprivan® after 12 hours because of the potential for  
microbial contamination and growth. Diprivan® must also be stored in a very narrow  
temperature range between 4 to 22°C. (Diprivan® Injectable Emulsion Product Insert,  
25 AstraZeneca (2001)).

PropoFlo™ Anesthetic Injection is an oil-in water emulsion containing, in addition to  
10 milligrams propofol per milliliter of emulsion, 100 mg soybean oil per mL, 22.5 mg  
glycerol per mL, 12 mg egg lecithin per mL, and sodium hydroxide. Like Diprivan®,  
PropoFlo™ is capable of supporting the growth of microorganisms. Failure to follow aseptic  
30 procedures may result in microbial contamination and associated medical complications.

Unused portions of PropoFlo™ must therefore be disposed of within 6 hours of vial entry (PropoFlo™ Anesthetic Injection Product Insert, Abbott Laboratories (1998)).

Rapinovet™ Anesthetic Injection is a white, oil-in-water emulsion containing, in addition to 10 milligrams propofol per milliliter of emulsion, 100 mg soybean oil/mL, 22.5 mg glycerol/mL, 12 mg egg lecithin/mL, 0.25 mg sodium metabisulfite/mL, and sodium hydroxide. Like Diprivan® and PropoFlo™, Rapinovet™ is capable of supporting the growth of microorganisms. (Rapinovet™ Anesthetic Injection Product Insert, Schering-Plough Animal Health (2000)).

GB-A-1,472,793 (see also, U.S. Patent Nos. 4,056,635; 4,452,817; and 4,798,846)

10 describes the use of propofol as an anesthetic and discloses certain injectable formulations of that compound. These formulations use a range of non-ionic surfactant concentrations with a water miscible, non-aqueous co-solvent such as an alcohol or glycol to solubilize an effective concentration of propofol. For example, one such formulation combines propofol with propylene glycol and a polyoxyethylene-polyoxypropylene block co-polymer known as  
15 Pluronic® F68 (Pluronic is a registered trademark used by BASF Corporation, Parsippany, New Jersey) in water. Pluronic® F68 is also commonly known as Poloxamer 188 or 'P188'. However, the use of propylene glycol and other water-miscible co-solvents in such formulations is associated with undesirable medical side effects such as concomitant pain on injection, superficial thrombophlebitis and intravasal haemolytic reactions.

20 International patent publication No. WO 01/64187 describes aqueous preparations of propofol that use poloxamer block co-polymer and are free of propylene and other non-aqueous co-solvents. However, the publication notes that the poloxamer P188 has a very limited ability to hold propofol (only 0.8% propofol in a 10% aqueous solution of P188). As a result, the formulations described in this publication must contain mixtures of P188 and  
25 another poloxamer compound such as P407. Yet only the poloxamer P188 has been approved by the U.S. Food and Drug Administration (FDA) for use in injectable formulations. Moreover, the use of poloxamers and other block copolymers at high levels can also be associated with undesirable side effects and is generally undesirable. See, for example, Blonder *et al.*, *Life Sci.* (1999) 65:PL261-266; and Johnston *et al.*, *J. Cardiovasc.*  
30 *Pharmacol.* (1999) 34:831-842.

International patent publication no. WO 00/78301 also describes aqueous formulations of propofol in P188 or P407. However, the formulations disclosed in this publication also contain an additional surfactant, such as Solutol® HS 15 (Solutol is a registered trademark used by BASF Corporation, Parsippany, New Jersey) or egg lecithin, or they contain co-solvents such as ethanol and/or polyethylene glycol. As noted above, however, the use of egg lecithin can support the growth of microorganisms, whereas co-solvents such as ethanol and polyethylene glycol can also be associated with undesirable side effects. Moreover, Solutol® is also associated with undesirable side effects and has not been approved for injectable formulations by the FDA.

Still other publications describe aqueous formulations that contain microemulsions of propofol in an oil or lipid. See, for example, International Patent Publication No. WO 00/10531. See also, U.S. Patent Nos. 6,140,374; 6,150,423; and publication no. US 2002/0120015 A1.

Hence, there is an ongoing need for formulations of propofol and, in particular, for injectable, aqueous formulations of propofol that are sterile and stable for indefinite periods under clinical conditions. At the same time, there is a need for aqueous formulations of propofol that minimize the use of surfactants, block copolymers, co-solvents and other excipients that may produce harmful or undesired side effects.

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The citation and/or discussion of a reference in this section and throughout the specification is provided merely to clarify the description of the present invention and is not an admission that any such reference is “prior art” to the invention described herein.

#### 4. SUMMARY OF THE INVENTION

The present invention relates to aqueous pharmaceutical compositions (also referred to herein as “formulations”) of therapeutic agents that are lipophilic and, consequently, not traditionally amenable to formulation in a homogenous, aqueous solution. More specifically, the invention relates to aqueous formulations of 2,6-diisopropylphenol (*i.e.*, propofol), its derivatives and analogues, and pharmaceutically acceptable salts thereof. It has been discovered that, by combining polyethylene glycol with a block co-polymer emulsifier, it is

possible to dissolve or suspend higher amounts of propofol and other lipophilic compounds while, at the same time, using smaller amounts of these excipients. As a result, it is possible to prepare stable, homogeneous aqueous formulations of propofol without using traditional emulsifiers, such as oils and/or lipids. The formulations of the invention are simple to  
5 prepare, are stable and can be readily stored for extended periods under a variety of different conditions. Moreover, because these compositions avoid the use of traditional emulsifiers which are substrates for bacterial growth, the compositions can be readily sterilized and can be preserved as sterile compositions for extended periods of time.

Preferred compositions of the invention are aqueous propofol formulations that  
10 comprise propofol in an aqueous medium with at least two excipients. Preferably, the two excipients are: (1) a block co-polymer, and (2) a polyethylene glycol (PEG). The block co-polymer can be, for example, a poloxamer such as poloxamer 188 (P188), poloxamer 407 (P407) or poloxamer 237 (P237). In preferred embodiments, the block co-polymer is P188. The polyethylene glycol can be a PEG having a molecular weight of 200 (PEG-200), 300  
15 (PEG-300), 400 (PEG-400), 600 (PEG-600), 800 (PEG-800) or 1000 (PEG-1000), with PEG-400 being particularly preferred.

Formulations of the present invention are able to contain higher levels of propofol in a homogeneous suspension or solution while, at the same time, using lower levels of excipients. Hence, preferred compositions contain at least 1% (w/v) and can have as much as  
20 5% (w/v) propofol. In particularly preferred embodiments, formulations of the invention comprise between about 1-2% (w/v) propofol with 1% (w/v) propofol being particularly preferred.

In various embodiments, the amount of block copolymer in a formulation of the invention is less than about 10% (w/v) of the formulation, with preferred amounts being  
25 between about 5 to 10% (w/v) of the formulation, and more preferably between about 6 to 8% (w/v) of the formulation. Preferred amounts of PEG in various embodiments of the invention are less than about 5% (w/v) of the formulation, and are more preferably between about 3 and 4% (w/v) of the formulation.

In other embodiments, formulations of the invention may comprise one or more  
30 additional excipients, such as tonicity modifiers, antimicrobial agents, and/or preservatives. For example, the invention provides aqueous propofol formulations that additionally

comprise a tonicity modifier selected from the group consisting of lactose, dextrose, dextrose anhydrous, mannitol, sodium chloride, potassium chloride, propylene glycol and glycerol. In a preferred embodiment, the tonicity modifier is propylene glycol, preferably in an amount that is not more than 5% (w/v) of the formulation, and more preferably not more than 2%

5 (w/v) of the formulation.

In still other embodiments, an aqueous propofol formulation of the invention can comprise a preservative, such as citric acid, preferably at a concentration between 2.5 and 15 mM, with a concentration of about 10 mM (*e.g.*, about 2.0 mg/ml) being particularly preferred. The invention provides additional embodiments in which an aqueous propofol

10 formulation comprises an antimicrobial agent. In certain such embodiments the antimicrobial agent can be selected from the group consisting of disodium edetate, metabisulfate, benzyl alcohol, cysteine or a salt thereof, and EDTA. In particularly preferred embodiments, the antimicrobial agent is benzyl alcohol, preferably in an amount of up to 0.5% (w/v) of the formulation.

15 In particular embodiments, the invention provides aqueous propofol formulations that comprise: (a) polaxmer 188 in an amount between 6 and 8% (w/v) of the formulation, (b) PEG-400 in an amount between 2 and 4% (w/v) of the formulation, (c) propylene glycol in an amount not greater than 2% (w/v) of the formulation, and (d) 2,6-diisopropylphenol (*i.e.*, propofol) in an amount between 1 and 2% (w/v) of the formulation. In such embodiments,

20 the formulations can also optionally comprise: (e) citric acid at a concentration between 2.5 and 15 mM (and more preferably about 10 mM or 2 mg/ml), and (f) benzyl alcohol in an amount up to 0.5% w/v (and more preferably 0.45% w/v) of the formulation.

Specific preferred embodiments of aqueous propofol formulations are also provided, including formulations as follows:

25 (1) P237 in an amount of about 3% (w/v) of the formulation, PEG-400 in an amount of about 6% (w/v) of the formulation, and propofol in an amount of about 1% (w/v) of the formulation;

(2) P188 in an amount of about 8% (w/v) of the formulation, PEG-400 in an amount of about 4% (w/v) of the formulation, propylene glycol in an amount of about 1% (w/v) of

30 the formulation, and propofol in an amount of about 1% (w/v) of the formulation;

(3) P188 in an amount of about 8% (w/v) of the formulation, PEG-400 in an amount of about 3% (w/v) of the formulation, propylene glycol in an amount of about 1% (w/v) of the formulation, and propofol in an amount of about 1% (w/v) of the formulation;

5 (4) P188 in an amount of about 8% (w/v) of the formulation, PEG-400 in an amount of about 4% (w/v) of the formulation, propofol in an amount of about 1% (w/v) of the formulation and substantially free of propylene glycol;

(5) P188 in an amount of about 8% (w/v) of the formulation, PEG-400 in an amount of about 3% (w/v) of the formulation, propofol in an amount of about 1% (w/v) of the formulation and substantially free of propylene glycol;

10 (6) P188 in an amount of about 7% (w/v) of the formulation, PEG-400 in an amount of about 4% (w/v) of the formulation, propylene glycol in an amount of about 1% (w/v) of the formulation, and propofol in an amount of about 1% (w/v) of the formulation;

15 (7) P188 in an amount of about 7% (w/v) of the formulation, PEG-400 in an amount of about 4% (w/v) of the formulation, propofol in an amount of about 1% (w/v) of the formulation and substantially free of propylene glycol;

(8) P188 in an amount of about 7% (w/v) of the formulation, PEG-400 in an amount of about 3% (w/v) of the formulation, propylene glycol in an amount of about 1% (w/v) of the formulation, and propofol in an amount of about 1% (w/v) of the formulation;

20 (9) P188 in an amount of about 7% (w/v) of the formulation, PEG-400 in an amount of about 3% (w/v) of the formulation, propofol in an amount of about 1% (w/v) of the formulation, and substantially free of propylene glycol;

(10) P188 in an amount of about 6% (w/v) of the formulation, PEG-400 in an amount of about 4% (w/v) of the formulation, propylene glycol in an amount of about 1% (w/v) of the formulation, and propofol in an amount of about 1% (w/v) of the formulation;

25 (11) P188 in an amount of about 6% (w/v) of the formulation, PEG-400 in an amount of about 4% (w/v) of the formulation, propylene glycol in an amount of about 2% (w/v) of the formulation, and propofol in an amount of about 1% (w/v) of the formulation;

(12) P188 in an amount of about 6% (w/v) of the formulation, PEG-400 in an amount of about 6% (w/v) of the formulation, propylene glycol in an amount of about 1% (w/v) of the formulation, and propofol in an amount of about 1% (w/v) of the formulation;

(13) P188 in an amount of about 8% (w/v) of the formulation, PEG-400 in an amount of about 4% (w/v) of the formulation, propofol in an amount of about 1% (w/v) of the formulation, and substantially free of propylene glycol; and

5 (14) P188 in an amount of about 9% (w/v) of the formulation, PEG-400 in an amount of about 2% (w/v) of the formulation, propofol in an amount of about 1% (w/v) of the formulation, and substantially free of propylene glycol.

In various other embodiments provided herein, any of the above-described formulations can additionally comprise citric acid, preferably at a concentration of between 2.5 and 15 mM and more preferably in an amount of about 20 mg per 10 milliliters of the 10 formulation (*i.e.*, a concentration of about 10 mM). Various embodiments are also provided in which any of the above-described formulations can additionally comprise benzyl alcohol, preferably in an amount of 0.45% (w/v) of the formulation.

In other aspects, the invention provides methods for administering propofol to a patient, and for either inducing or maintaining anesthesia in a patient. These methods 15 involve administering any of the above described aqueous propofol formulations of the invention to the patient, until an effective amount of propofol (*e.g.*, effective for inducing or maintaining anesthesia) has been administered.

## 5. BRIEF DESCRIPTION OF THE DRAWINGS

20 **Figure 1** illustrates exemplary data from HPLC analysis of aqueous propofol preparations described in the examples, *infra*, that are stored for a period of 1, 2, 3 and 4 weeks at 80 °C. Data from HPLC analysis of the following formulations is shown: M841 (1% propofol, 8% poloxamer 188, 4% PEG-400 and 1% propylene glycol), M831 (1% propofol, 8% poloxamer 188, 3% PEG-400 and 1% propylene glycol) and M731 (1% 25 propofol, 7% poloxamer 188, 3% PEG-400 and 1% propylene glycol). All samples contain citric acid (2 mg/ml) and benzyl alcohol (0.45%). Percentages of different excipients and other ingredients are in weight/volume.

30 **Figures 2A-2B** illustrate data from exemplary HPLC analysis of aqueous propofol preparations referred to in the examples, *infra*, as M831 (1% propofol, 8% poloxamer 188, 3% PEG-400 and 1% propylene glycol) that are stored at 80 °C under a variety of different

pH conditions. **Figure 2A** shows data from samples stored at pH 5.0, 6.0 and 7.0 for 1, 2, 3 and 4 weeks. **Figure 2B** shows data from samples stored at pH 6.0, 6.2, 6.4, 6.5 and 6.6. All samples contain 0.45% benzyl alcohol and 2 mg/ml citric acid. Percentages of different excipients and other ingredients are in weight/volume.

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**Figure 3** illustrates data from exemplary HPLC analysis of aqueous propofol preparations referred to in the examples, *infra*, as M831 (1% propofol, 8% poloxamer 188, 3% PEG-400 and 1% propylene glycol) stored at 80 °C and containing various concentrations of citric acid (0.5, 1 and 2 mg/ml). All samples contain 0.45% benzyl alcohol.

10 Percentages of different excipients and other ingredients are in weight/volume.

**Figure 4** illustrates data from exemplary HPLC analysis of aqueous propofol preparations described in the examples, *infra*, and referred to as M831 and M830. Samples are analyzed after storage at 80 °C, pH 5.0 for 0, 1, 2 and 3 weeks. The extent of propofol degradation in these samples is estimated from the area under elution curves for the particular impurity 3,3',5,5'-tetraisopropyl-4,4'-dihydroxybiphenyl (Impurity E) and by estimating the total impurity level of the preparations. M831 (1% propofol, 8% poloxamer 188, 3% PEG-400 and 1% propylene glycol, 0.45% benzyl alcohol and 2 mg/ml citric acid), M830 (1% propofol, 8% poloxamer 188, 3% PEG-400 and 0% propylene glycol, 0.45% benzyl alcohol and 2 mg/ml citric acid). Percentages of different excipients and other ingredients are in weight/volume.

**Figure 5** illustrates the preferred chemical structure of 2, 6-diisopropylphenol (Formula I), which is also known as propofol. In propofol, R is a hydrogen (H). Formulas (II) through (VII) should chemical structures for other chemical moieties which can be substituted for R in some preferred derivatives and/or analogues of propofol.

## 6. DETAILED DESCRIPTION

The present invention relates to aqueous formulations of a lipophilic therapeutic agent. In preferred embodiments, the application relates to aqueous formulations of 2,6-diisopropylphenol, which is also commonly known as propofol. For convenience,

formulations are described here as containing propofol as the active ingredient. However, formulations of the invention can actually contain any active ingredient or any combination of active ingredients. In preferred embodiments, a formulation of the invention will contain at least one active ingredient that is lipophilic and, consequently, not ordinarily normally used in aqueous formulations. For example, traditional formulations of propofol comprise oil or lipid emulsions since that compounds lipophilic nature has made it difficult to store in homogenous, aqueous formulations.

Propofol is understood to have the chemical structure of Formula (I) set forth in **Figure 5**, where the moiety R is H. However, derivatives and analogues of propofol are also known and can be used in formulations of the present invention. Hence, the terms “2,6-diisopropylphenol” and “propofol,” when used to describe the present invention, refer to the compound set forth in Formula (I) of **Figure 5** as well as to derivatives, analogues thereof. The terms “2,6-diisopropylphenol” and “propofol” also refer, in the context of the present invention, to pharmaceutically acceptable salts of propofol, its derivatives and analogues.

Examples of propofol analogues and derivatives that can be used in the present invention include compounds having the chemical structure set forth in Formula I (**Figure 5**) where the moiety R has the chemical structure set forth in any of Formulas (II), (III), (IV), (V), (VI) and (VII) in **Figure 5**. Preferred propofol analogues and derivatives include compounds of Formula (I) in which R has the formula set forth in Formula (II), where x is 2 and the propofol analogue is a hemisuccinate ester of propofol. As another example, R can have the chemical structure set forth in Formula (II), where x is 4 and the propofol analogue is a hemiadipate ester of propofol. Alternatively, R can have the chemical structure set forth in Formula (V), where R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> can be either the same or different chemical moieties and are independently selected from the group consisting of hydrogen, an alkyl, an alkenyl, an alkynyl, an alkoxy, an allenyl, an aryl, an aralkyl, or a halo or halogen. These moieties (*i.e.*, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub>) can be substituted or heteroatom containing. Alternatively, R can have the chemical structure set forth in Formula (VI) where Y is a phosphono protecting group which can be benzyl, t-butyl, or an allyl group. In other embodiments R can have the chemical structure set forth in Formula (VII) where Z is hydrogen or a metal or amine that forms pharmaceutically acceptable salts.

Pharmaceutically acceptable salts include all pharmaceutically acceptable salts of propofol or any of its derivatives or analogues. Hence, “pharmaceutically acceptable salts” include those salts of propofol, its derivatives or analogues that retain the biological effectiveness and properties of the free acids or free basis and that are not otherwise unacceptable for pharmaceutical use. Pharmaceutically acceptable salts of propofol derivatives include, for example, salts of acidic or basic groups which may be present in a propofol derivative. Derivatives of propofol that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that can be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts; *i.e.*, salts containing pharmacologically acceptable anions such as chloride, bromide, iodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, pantothenate, bitartrate, ascorbate, succinate, maleate, bentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzensulfonate, *p*-toluenesulfonate and pamoate (*i.e.*, 1, 1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Derivatives of propofol that include an amino moiety can also form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above. Derivatives of propofol that are acidic in nature are capable of forming a wide variety of salts with various inorganic and organic bases. Suitable base salts are formed from bases that donate cations to form non-toxic salts. Suitable cations include, but are not limited to, sodium, aluminum, calcium, lithium, magnesium, potassium, zinc, and diethanolamine salts. A more comprehensive review of pharmaceutically acceptable salts that can also be used in the present invention is provided by Berge *et al.* (*J. Pharm. Sci.* (1977) 66:1-19).

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#### 6.1. Propofol Formulations

The terms “composition” and “formulation” are used interchangeable herein and refer to mixtures that comprise propofol (as an active ingredient) and at least one excipient. Preferred formulations of the invention comprise propofol and at least two excipients, which are preferably a block copolymer and a polyethylene glycol (PEG). The terms “excipient” and “additive” are used interchangeably to describe the present invention, and refer to any

compound contained in a formulation other than its primary activity ingredient (*i.e.*, other than propofol) or water. Excipients can be inert or they can chemically or physically affect other composition components. In addition, an excipient or additive may have other properties of its own, for example, as a stabilizer or anti-microbial agent. Excipients can 5 include, but are not limited to, surface active agents (for example, surfactants, emulsifiers, detergents, binders and wetting agents), salts, polymers, solvents, antimicrobials, preservatives, fillers, diagnostic agents, sugars, alcohols, acids, bases and buffers.

It is noted that exemplary, preferred amounts or ranges of amounts for propofol, excipients and various other ingredients in the formulations of this invention are provided 10 throughout the description of this invention and its various embodiments. However, it will be appreciated by those skilled in the art that the precise amount of an ingredient used is not critical for practicing the invention. Rather, the amount specified for any ingredient in the description of this invention is merely approximate. Formulations containing about the same amount of a particular ingredient can also be used, even when the words “about” and/or 15 “approximate” are not used here to describe the preferred amount of that ingredient.

Generally, the amount used can be within 25% of an amount specified herein, although the amount used is more preferably within 15%, 10%, 5%, 2% or 1% of the amount specified.

As noted above, formulations of the present invention preferably comprise at least 20 two excipients which are present, along with propofol, in a homogenous aqueous phase. In preferred embodiments, the two excipients are: (1) a block co-polymer, and (2) a polyethylene glycol (PEG). In particular, it has been discovered that the inclusion of a small amount (*e.g.*, between 2 and 6%) of a polyethylene glycol with a block co-polymer surfactant produces a synergistic effect, greatly increasing the amount of a lipophilic active ingredient 25 such as propofol that can be homogenously suspended in an aqueous medium. As a result, the total amount of excipients used in a formulation of the invention is significantly reduced compared to amounts that have been traditionally used in propofol preparations.

Generally, the concentration of excipients should be as low as possible, to minimize 30 the risk of undesired excipient effects (see, for instance, Blonder *et al.*, *Life Sci.* (1999) 65:PL261-266; and Johnston *et al.*, *Cardiovasc. Pharmacol.* (1999) 34:831-842). In preferred embodiments, therefore, the concentration of excipients in a formulation is less

than about 50%, and is more preferably less than about 40%, less than about 30%, less than about 25%, less than about 20%, less than about 15% or even less than about 10% (w/v).

For example, in preferred embodiments that total amount of block co-polymer in a formulation is less than about 10% (w/v) of the formulation, and is more preferably between 5 about 5 and 10% (w/v). In particularly preferred embodiments, the total amount of block co-polymer is a formulation of the invention is between about 6 and 8% (w/v) of the formulation.

Preferably, the block co-polymer used in a formulation of the present invention is a “poloxamer”. Poloxamers are poly(a-oxyethylene-b-oxypropylene-a-oxyethylene) triblock 10 copolymers that are commonly used as surfactants and are generally regarded as non-toxic. The solubility of poloxamers in water is generally good. However, the properties of individual poloxamers can vary substantially. Poloxamer copolymers can be obtained from BASF Corporation (Parsippany, New Jersey) under the Pluronic® registered trademark. Preferred poloxamers that can be used in formulations of the invention include poloxamer 15 124 (P124), poloxamer 188 (P188), poloxamer 237 (P237), poloxamer 338 (P338) and poloxamer 407 (P407), although any poloxamer can be used. In addition poloxamers as described in U.S. Patent No. 5,990,241 can also be used in formulations of the invention. In preferred embodiments, a poloxamer used in formulations of the present invention is one approved (for example, by the U.S. Food and Drug Administration) for use in pharmaceutical 20 preparations (*e.g.*, for administration to humans) and, in particular approved for use in intravenous and/or other injectable formulations. At present, only poloxamer 188 is believed to be approved for such uses in the United States. Hence, poloxamer 188 is particularly preferred in formulations of the present invention at this time. However, other poloxamers, such as P237, may actually be superior solubilizers of lipophilic compounds such as 25 propofol. It is therefore expected that such other poloxamer compounds will be preferred for use in the present invention should they also be approved for use in injectable preparations, *e.g.*, for intravenous administration.

Purified poloxamers with narrower ranges of polymer molecular weight composition can be selected for a composition of this invention. Narrower ranges (*e.g.* compared to 30 commercially available poloxamer 188 or poloxamer 237) of purified poloxamer may have a polydispersity value of, for example, 1.01, 1.02, 1.04, 1.05, 1.1, 1.3, 1.5, 2, 3, or 4. In some

embodiments, the polydispersity value of a purified poloxamer is between 5 and 1, between 4 and 1, between 3 and 1, between 2 and 1, between 1.5 and 1, between 1.3 and 1, between 1.2 and 1 or between 1.1 and 1. In some embodiments, a purified poloxamer contains at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of polymers with

5 a molecular weight between 2000 and 15,000. In some embodiments, a purified poloxamer contains at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of polymers with a molecular weight between 3000 and 14,000. In some embodiments, a purified poloxamer contains at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of polymers with a molecular weight between 4000 and 13,000. In

10 some embodiments, a purified poloxamer contains at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of polymers with a molecular weight between 5000 and 12,000. In some embodiments, a purified poloxamer contains at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of polymers with a molecular weight between 5000 and 11,000. In some embodiments, a purified poloxamer contains at least

15 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of polymers with a molecular weight between 6000 and 10000. In some embodiments, a purified poloxamer contains at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of polymers with a molecular weight between 7000 and 9000. In some embodiments, a purified poloxamer contains at least 50%, at least 60%, at least 70%, at least 80%, at least

20 90%, or at least 95% of polymers with a molecular weight between 7500 and 8500. In some embodiments, a purified poloxamer contains at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of polymers with a molecular weight between 8000 and 9500.

25 Preferred formulations of the invention also comprise a polyethylene glycol (PEG) in combination with the poloxamer or other block co-polymer. In particular, it has been discovered that the combination of PEG with block co-polymers greatly increases the amount of propofol that can be held in an aqueous phase suspension, solution or emulsion. Consequently, aqueous formulations of propofol that are suitable for injection can now be

30 made without the use of oil or lipid based emulsions. Preferably, the amount of PEG in a formulation of the invention is not greater than about 10% (w/v) of the formulation, and more

preferably is not greater than about 5% (w/v) of the formulation. In various embodiments, however, the total amount of PEG in a propofol formulation of the invention can be as high at 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% (w/v) of the formulation.

Particularly preferred formulations of the invention comprises between about 2% and about 5% 6% (w/v) PEG and, more preferably, between about 2 and 4% PEG. Polyethylene glycols having a molecular weight of about 200 (*i.e.*, PEG-200), 300 (PEG-300), 400 (PEG-400), 600 (PEG-600), 800 (PEG-800) or 1000 (PEG-1000) can be used. However, the use of PEG-400 is preferred.

It has been discovered that, by combining PEG with a block co-polymer surfactant such as a poloxamer, it is possible to greatly increase the amount of propofol or other lipophilic active ingredient that is held in a homogeneous, aqueous phase (*e.g.*, in a solution, suspension or emulsion) without requiring the use of traditional oil or lipid excipients. Hence, formulations of the present invention can also contain higher levels of propofol than traditional formulations. Thus, for example, formulations of the present invention can comprise an amount of propofol as high as 10% (w/v) of the formulation, although amount of 15% (w/v) or less are generally more preferred. Such higher amounts of propofol will require the administration of smaller volumes to achieve the same therapeutic effect, and are generally more cumbersome or difficult to safely administer. Hence, more preferred formulations of the invention comprise propofol in an amount that is not greater than about 20% 2% (w/v) of the formulation. Preferred formulations comprise propofol in an amount between 0.5 and 2% (w/v) of the formulation, and more preferably between 1 and 2% (w/v) of the formulation, with amount of about 1% (w/v) being particularly preferred.

Propofol compositions of the present invention can further comprise other ingredients, that are not normally considered excipients and which may also be biologically active. For example, a propofol composition of the invention may comprise one or more additional anesthetic and/or antioxidative agents. Alternatively, propofol formulations of the invention may be co-administered with one or more such additional active agents.

30 Pharmaceutical compositions that are intended for application to delicate membranes of the body are commonly adjusted to approximately the same tonicity (*i.e.*, isotonicity) as

that of the body fluids. Isotonic compositions are those that cause minimal swelling or contraction of the tissues with which they come in contact, and produce little or no discomfort when instilled in body tissues. Preferably, the propofol compositions are substantially isotonic. The compositions may additionally comprise one or more tonicity modifiers. Examples of tonicity modifiers include, but are not limited to, lactose, dextrose, dextrose anhydrous, mannitol, sodium chloride, potassium chloride, propylene glycol and glycerol.

The use of propylene glycol as an isotonic agent in the present invention is particularly preferred. Hence, preferred formulations of the invention comprise propylene glycol in addition to the block co-polymer and polyethylene glycol excipients described *supra*. Generally, the amount of propylene glycol in a formulation of the present invention will not be greater than about 5% (w/v) of the formulation, with amounts less than or equal to about 2% being preferred. Preferred formulations of the invention comprise propylene glycol in an amount of about 0%, 1% or 2%.

15

Although not necessary to practice the present invention, preferred aqueous propofol compositions can also comprise an optional antimicrobial agent. For example, a formulation of the invention can comprise disodium edetate, metabisulfate, or a preservative such as benzyl alcohol, or an antioxidant such as cysteine or a salt thereof to retard the growth of microorganisms. In this embodiment, the compositions of the present invention comprise a microbiostatic, microbicidal, preservative, or antioxidant (e.g., cysteine or a salt thereof) in a concentration sufficient to exhibit microbiostatic or microbicidal activity against those microorganisms most likely to contaminate the propofol compositions. A further embodiment includes a sterile pharmaceutical composition for parenteral administration which comprises an aqueous solution of propofol, and which further optionally comprises a microbiostatic, microbicidal, preservative, or antioxidant such as cystein (or a salt thereof), EDTA, benzyl alcohol, or metabisulfite, and wherein said aqueous propofol solution is sufficient to prevent no more than a 10-fold increase in growth, or will support no more than a 10-fold increase in growth, of each of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231 for at least 24 hours as measured by a test wherein a washed suspension of each said organism is

added to a separate aliquot of said composition at approximately 50 colony forming units per ml, at a temperature in the range 20°C to 25°C., whereafter said aliquots are incubated at 20°C to 25°C for 24 hours and thereafter tested for viable counts of said organism. Another embodiment includes a method for producing anaesthesia in a warm-blooded animal which

5 comprises parenterally administering to said animal in need thereof an anaesthetically effective amount of a sterile pharmaceutical composition which comprises an aqueous solution of propofol, and which composition further optionally comprises a microbiostatic, microbicidal, preservative, or antioxidant such as cystein (or a salt thereof), EDTA, or metabisulfite, and wherein said aqueous propofol solution is sufficient to prevent no more

10 than a 10-fold increase in growth, or will support no more than a 10-fold increase in growth, of each of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231 for at least 24 hours as measured by a test wherein a washed suspension of each said organism is added to a separate aliquot of said composition at approximately 50 colony forming units per ml, at a

15 temperature in the range 20°C to 25°C., whereafter said aliquots are incubated at 20°C to 25°C for 24 hours and thereafter tested for viable counts of said organism.

In addition, preferred formulations of the invention can also comprise citric acid or a salt thereof. Without being held to any particular theory, Applicants believe that citric acid

20 or a salt thereof in the compositions of the present invention exhibits antioxidant and/or chelating properties. Applicants have discovered that compositions comprising citric acid or a salt thereof possess an unexpectedly high degree of propofol stability. Also, Applicants have discovered that compositions comprising ascorbic acid or salts thereof unexpectedly display significant propofol degradation. Thus, citric acid or a salt thereof is added to the

25 compositions of the present invention for its favorable effects including but not limited to modifying pH and/or providing or enhancing (a) antioxidant characteristics, (b) chelating effects of the composition, and/or (c) stability of the excipient(s) or the active agent(s) such as, for example, the propofol compound. Citric acid or a salt thereof is preferably present in a concentration sufficient to optimize and balance the desired pH and/or the desired antioxidant

30 or chelating properties. In one aspect, the present invention is directed to propofol containing compositions wherein the composition further comprises citric acid or a salt or salts of citric

acid in a concentration of at least about 0.05 percent (w/v), in particular, as at least about 0.1 percent (w/v). For example, citric acid or one or more salts thereof can be present in a formulation of the present invention in an amount between about 0.05 and 3%, with amounts between about 0.05 and 0.2% being particularly preferred. In preferred embodiments, citric  
5 acid can be present in a formulation of the invention at a concentration of between about 2.5 and 15 mM, with concentrations of about 10 mM (*i.e.*, about 2 mg/ml) being particularly preferred.

In some embodiments, excipients are present in the propofol containing compositions  
10 in the lowest concentrations that will support the formation of a stable composition (e.g., a physically, thermodynamically, and/or chemically stable composition). Keeping excipient concentrations as low as possible helps to minimize the risk of undesired excipient effects. The propofol containing compositions are preferably free of preservatives and/or anti-microbials. Preferably, the compositions are also sterile and pyrogen-free.

15 Preferred formulations of the invention are also substantially free of lipid components such as lecithin, castor oil, soybean oil, phospholipids, fatty acids, triablycerols, *etc*, that are commonly used in traditional propofol formulations. As such, the preferred formulations of this invention do not support microbial growth, or the rate of microbial growth in these compositions is significantly slower when compared to such growth in conventional, lipid-  
20 containing formulations.

The term “substantially free,” as used herein, refers to compositions that contain the indicated component in only minor amounts if at all. For example, a composition of the invention can be “substantially free” of an ingredient and still contain trace amounts of that component, *e.g.*, as a degradation process. Preferably, however, compositions of the  
25 invention that are “substantially free” of a certain component will contain that component only a minimal concentration, for example, of less than about 3%, more preferably less than about 1%, less than about 0.5%, or less than about 0.1%. Even more preferably, a composition that is said to be “substantially free” of some component will contain less than about 0.05% (w/v) or even less than about 0.01% (w/v) of that component. In fact,  
30 particularly preferred compositions that are “substantially free” of a particular component will not contain measurable or detectable amounts of that component.

Preferred compositions of the invention have substantially reduced concentrations of excipients that promote and/or facilitate microbial growth compared to traditional emulsions and other formulations of propofol. Indeed, particularly preferred propofol formulations of the invention are substantially free of and/or do not contain any such excipients. These

5 include excipients, such as lipids and/or oils, that are traditionally used to solvate or suspend propofol in aqueous formulations. In addition, compositions of the invention are preferably free of or are substantially free of esters of medium or long chain fatty acids (e.g., about C<sub>6</sub> to about C<sub>25</sub> fatty acids) such as glyceryl esters of medium or long chain fatty acids (e.g., mono- di- or triacylglycerols). Preferably, the compositions of the invention are also substantially

10 free of triaglycerols such as, for example, those contained in vegetable oils (e.g., soybean, castor, sunflower, and artichoke oils). In another embodiment, the compositions are free of or are substantially free of phospholipids (e.g., naturally occurring phospholipids or phospholipids that are synthetically produced and modified).

15 Examples of some preferred formulations in the present invention are described in the Examples, *infra*, including the formulations set forth in Table I of those examples. Of these, the formulation identified as M831 (1% propofol, 8% poloxamer 188, 3% PEG-400, and 1% propylene glycol) is particularly preferred. Any of these or other formulations of the invention may additionally comprise, e.g., a preservative such as citric acid (as described above) and/or an antimicrobial agent such as benzyl alcohol (as also described above).

20 Preferred formulations of the invention do comprise citric acid, preferably at a concentration of between about 2.5 and 15 mM and with a concentration of 20 mg per 10 milliliters of formulation (*i.e.*, about 10 mM) being particularly preferred. Preferred formulations of the invention also contain benzyl alcohol in addition to the other, above-described excipients.

25 Preferably, benzyl alcohol is present as an antimicrobial agent and in an amount of about 0.45% (w/v) of the formulation.

Aqueous formulations of the invention are preferably clear, transparent and sterile, or they can be readily sterilized by conventional and routine methods such as ultrafiltration.

30 Moreover, formulations of the invention are both chemically and physically stable over a wide

range of environmental conditions, including a wide range of different temperature and pH conditions.

Compositions of the present invention can also be characterized, *inter alia*, by their macroscale homogeneity. Macroscale homogenous compositions are characterized by a lack 5 of distinguishable phase separation. Conventional propofol emulsions are milky white in appearance, which indicates that presence of two or more distinct phases such as oil droplets suspended in an aqueous phase (*i.e.*, water). The oil droplets in such emulsions, which are typically about one micron in size, scatter light giving rise to their milky or “hazy” appearance.

10 By contrast, compositions of the present invention are preferably clear or transparent to the naked eye. Without being limited to any particular theory or mechanism of action, it is believed that this visual clarity indicates that propofol in such compositions is suspended in particles that are substantially smaller than in conventional formulations, *e.g.*, oil-in-water emulsions, of propofol. For instance, the examples, *infra*, describe dynamic light scattering 15 and/or other measurements indicating the presence of nano-scale particles in compositions of the present invention, such that light scattering is minimized and the system appears as a homogeneous composition.

It is believed that compositions that maintain clarity under visual inspection over time (including propofol formulations of the present invention) possess a greater degree of 20 thermodynamic stability than that possessed by conventional emulsions. Generally, solutions or mixtures having a high degree of thermodynamic stability tend to maintain particles or particle agglomeration in solution or to preserve their suspension in a liquid over significant periods of time and/or under conditions that do not typically favor continued solvation or suspension. For example, solutions or suspensions that are not thermodynamically favored 25 will typically exhibit a separation of phases such as a precipitation of solute or suspended matter. Environmental conditions can be selected to maintain thermodynamically disfavored states over longer periods of time. For example, refrigeration is often used to help maintain the suspension of particles in an emulsion.

Typically, compositions of the present invention maintain the solvation and/or 30 suspension of their component ingredients over long periods of time (*e.g.*, for at least as long as conventional oil-in-water propofol emulsions) and/or under conditions more unfavorable to

thermodynamic stability (*e.g.*, at higher temperatures and/or under harsher pH conditions). For example, compositions of the invention preferably exhibit visual clarity to the naked eye even when stored at elevated temperatures over extended periods of time. Phase separation of compositions can also be assessed microscopically, by light scattering or nephelometry, or by 5 other suitable methods that are well known to those of ordinary skill in the art. For instance, and not by way of limitation, the examples, *infra*, describe experiments demonstrating that aqueous propofol formulations of the invention are stable for periods of four weeks or more, even when stored under very harsh environmental conditions, *e.g.*, of elevated temperature (80 °C) and/or pH. However, it will be appreciated that formulations of the invention will 10 remain stable for even greater periods of time, particularly when stored under more favorable conditions. For example, formulations of the invention are expected to remain stable for periods of up to 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, six months, eight months, ten months, twelve months, eighteen months or more. In fact, compositions of the invention can even be stored for periods of up to 1, 2, 3, 5, 10 or more years and still maintain their solvation 15 or suspension, particularly if stored at a temperature between about 10 and 40 °C and more preferably at a temperature between about 15 and 25 °C. Preferably, the compositions are also stored at a pH of about 5.0 ± 0.5. Alternatively, compositions of the invention may also be refrigerated stored for the above-listed time periods or greater. Although compositions of the invention may become hazy (indicating a loss of suspension or solvation) when stored under 20 such conditions, data presented in the examples, *infra*, demonstrate that this loss of suspension/solvation is reversible. Hence, compositions stored under such conditions can become clear and/or transparent again once equilibrated to room temperature, indicating resolvation or resuspension of the lipophilic propofol component.

The compositions of the present invention can be characterized by the size of the 25 particles (mean diameter) present in the composition. Without being held to any particular theory, it is believed that in some embodiments the particles contained in the compositions take the form of micelles of various sizes. Alternatively, it is believed that some compositions, or portions of compositions, take the form of micro- or nano-emulsions. The particle size, also herein referred to as the particle geometric size or particle geometric diameter, can be determined using any of the techniques known to those of skill in the art. 30 For example, a Malvern Instruments Zetasizer can be used to determine the size of particles

in a composition. The Zetasizer line of measurement systems uses the technique of Photon Correlation Spectroscopy (PCS) to measure submicron particle size. Particles dispersed in a fluid are in constant random motion, commonly referred to as Brownian motion. Photon Correlation Spectroscopy measures the speed of this motion, calculates the diffusion speed of  
5 the particles, and relates this to particle size using the Stokes-Einstein equation. One skilled in the art also may employ other suitable means to determine particle size.

In addition to Photon Correlation Spectroscopy (PCS), other methodologies relating to particle size analysis known to those skilled in the art can be employed including, but not limited to, microscopy (e.g., optical and electron), electrozone or photozone sensing, and  
10 other light scattering techniques (e.g., laser diffraction).

In some embodiments, the compositions have an average particle size (mean diameter) less than about 100 nanometers, between about 10 and about 100, between about 25 and about 90 nanometers, or between about 30 and about 75 nanometers. Compositions of the invention consist of particles having a geometric diameter of less than about 90, less than  
15 about 75 nanometers, less than about 65 nanometers, less than about 55 nanometers less than about 50 nanometers, less than about 45 nanometers, less than about 40 nanometers, less than about 35 nanometers, less than about 30 nanometers, less than about 25 nanometers, less than about 20 nanometers, less than about 15 nanometers, less than about 10 nanometers, less than about 5 nanometers, or less than about 1 nanometer. In some embodiments, the compositions  
20 have an average particle size of between about 50 and 250 nanometers, between about 50 and 150 nanometers, between about 150 and 250 nanometers, and between about 100 and 200 nanometers. In some compositions, all particles have a relatively similar particle size. A relatively similar particle size is defined as the particle size and consistency required of a pharmaceutical product to attain US Food and Drug Administration human drug approval.  
25 Optionally, compositions of the present invention are filtered to produce compositions comprising particles of desired sizes or average sizes. Methods for filtering such compositions are well known to those skilled in the art.

In some embodiments, the compositions of this invention have superior clinical benefits compared to currently marketed propofol formulations or other aqueous propofol  
30 formulations. Superior clinical benefits can include, but are not limited to, decreased lipid

levels, faster onset of action, faster offset of action, decreased damage to red blood cells, and fewer side effects.

The compositions of the invention can be characterized by the chemical stability of the therapeutic, prophylactic or diagnostic agents that comprise the particles. The chemical 5 stability of a constituent anesthetic agent can affect important characteristics of a pharmaceutical composition including shelf-life, proper storage conditions, acceptable environments for administration, biological compatibility, and effectiveness of the agent. Chemical stability can be assessed using techniques well known in the art. For example, assays to detect degradation information obtained from stress studies (e.g., products of acid 10 and base hydrolysis, thermal degradation, photolysis, and oxidation) for both active ingredients and excipients are numerous. One example of a technique that can be used to assess chemical stability is reverse phase high performance liquid chromatography (HPLC).

The compositions of the invention do not exhibit substantial propofol degradation such as, for example, no more than about 5% or no more than about 3% loss of propofol 15 potency at room temperature over a given study period. Alternatively, propofol degradation can be assessed by measuring propofol degradate concentrations such as, for example, quinone and dimer concentrations. In some embodiments, the compositions do not exhibit substantial increases in propofol degradates such as, for example, no more than about 0.05%, no more than about 0.1%, or no more than about 0.2% increase in propofol degradate 20 concentration over a given study period. In a preferred embodiment, any single degradate does not exceed the International Conference on Harmonization (ICH) guidelines, unless specific qualification of that degradate has been performed. (See ICH Document Q3B).

In one embodiment, the compositions do not experience substantial propofol degradation for a period of at least about 6 months when stored refrigerated. Preferably, the 25 compositions do not experience substantial propofol degradation for a period of at least about one year when stored refrigerated. Even more preferred, the compositions do not experience substantial propofol degradation for at least about 6 months, for at least about one year, or, most preferably, for at least about two years when stored at or below about room temperature.

30 The compositions of the present invention preferably have a physiologically neutral pH, such as between about 5 and about 9. The pH of the propofol containing compositions can

be adjusted as necessary by, for example, the addition of a base or a salt thereof, for example, an alkali such as sodium hydroxide, potassium hydroxide, or the like. Alternatively, an acid or a salt thereof such as hydrochloric acid, citric acid, or the like can be used to adjust the pH of the compositions. The term “pH modifier,” as used herein, refers to substances such as acids, 5 bases, or salts thereof that are used to adjust the pH of a composition and that are well known to those skilled in the art.

In some embodiments, the stability of the compositions of this invention are sensitive to pH. In some compositions, propofol containing compositions have greater stability at a pH of about 5 to 6, at about 4.5 to 6.5, at about 4.5 to 5.5, at about 5 to 7.5 at about 6 to 7, or at 10 about 6.5 to 7.5. The pH of the composition can be adjusted with a pharmaceutically acceptable acid or base to obtain a desired pH. In some embodiments, a specific pH can affect the composition stability or microbial growth.

## 6.2. Methods of Manufacture

15 The propofol containing compositions are preferably provided or administered as sterile pharmaceutical compositions. For example, the propofol containing compositions are administered substantially free of microorganisms. The preparation of sterile pharmaceutical compositions is well known to those experienced in the art. Sterile propofol containing compositions can be prepared using conventional techniques such as, for example, 20 sterilization of final products or aseptic manufacture. In a preferred embodiment, the sterile compositions of the invention are substantially free of microorganisms for a longer period of time after opening than currently available propofol compositions such as Diprivan® Injectable Emulsion.

The compositions of the present invention can be provided in forms that possess 25 desired propofol concentrations and are ready for direct administration to a patient. Alternatively, compositions can be provided in a concentrated form that requires dilution, for example, with water or an injectable solution, prior to administration. In the case of intravenous administration, the compositions can be admixed with diluents suitable for intravenous administration well known to those experienced in the art. Such diluents include 30 water and injectable, aqueous sodium chloride and dextrose solutions. Due to the clear and

homogenous character of the compositions of the invention, if further diluted, the resulting diluted compositions are generally also homogeneous and clear.

Compositions of the present invention can be formed by mixing 2,6-diisopropylphenol, one or more excipients, and water. Various methods of mixing the composition components are contemplated. Excipients can be mixed into the compositions as neat excipients or as excipients in water. Propofol can be mixed into at least one or more neat excipients or into at least one or more excipients in water. The 2,6-diisopropylphenol may be mixed with at least one or more excipients in water and then combined with either (1) at least one or more neat excipients or (2) with at least one or more excipients mixed in water. In a preferred embodiment, the excipients are mixed together, water is added with mixing, then propofol is added with mixing, and finally, additional water is optionally added to increase the mixture volume. Also preferred, excipients in water are mixed together, propofol is added with mixing, and finally, additional water is optionally added to increase the mixture volume. In most embodiments, propofol is added last.

The water used in the compositions of the present invention is preferably suitable for animal, including human, injection. The water should meet appropriate government and/or health care industry standards. Preferably, the water meets United States Pharmacopeia (USP) 23 standards for Pharmaceutical Grade Water for Injection. Normally, the water should contain no added substances.

Mixing may be performed by any of the various methods known in the art. A mixing apparatus may be batch or continuous. Examples of suitable mixing apparatuses include jet mixers, injectors, mixing nozzles, pumps, agitated line mixers, packed tubes, gas agitated vessels, and stirred vessels, among others. Mixing can be carried out at any temperature that does not substantially degrade the composition components. Typically, mixing is performed at or near room temperature. An advantage of practicing the present invention is the ease by which the compositions can be prepared compared with the methods, such as, for example, microfluidization techniques, often necessary to form propofol compositions, for example, conventional propofol emulsions.

The compositions can be provided, prepared, stored, or transported in any container suitable for maintaining sterility. The container can incorporate means for dispensing an aqueous composition such as, for example, a pierceable or removable seal. The compositions

can be dispensed, for example, by extraction with a syringe or by pouring the composition directly into a device (e.g., a syringe, intravenous (IV) bag, or machine) for administration to a patient. Other means for providing, preparing, storing, transporting, and dispensing sterile pharmaceutical compositions are known to those skilled in the art.

5 In one embodiment, the compositions of the invention are manufactured, packaged, stored, or administered under an oxygen free atmosphere since 2,6-diisopropylphenol is subject to oxidative degradation. Oxygen free atmospheres include nitrogen, argon, or krypton gas, among others. Preferably, the compositions are manufactured, packaged, and stored under a nitrogen gas atmosphere.

10

### 6.3. Uses of Propofol Formulations

The present invention is also directed to methods of administering 2,6-diisopropylphenol to a subject in need of anesthesia, the methods comprising intravenously delivering to the subject a sterile pharmaceutical composition. Sterile pharmaceutical compositions acceptable for delivery to a subject are described herein. In one embodiment, a method is provided for administering 2,6-diisopropylphenol to a subject in need of anesthesia comprising intravenously delivering to the subject a sterile pharmaceutical composition comprising 2,6-diisopropylphenol, and one or more excipients; wherein the composition is substantially free of triacylglycerols. The composition also can be substantially free of other glyceryl esters of medium or long chain fatty acids or phospholipids as described herein.

20 The compositions of the present invention can be administered to a patient for the induction and/or maintenance of anesthesia, by administering an amount of the composition to a patient so that the patient receives an amount or dose of propofol that is effective for either inducing or maintaining anesthesia. The use of propofol as an anesthetic is known.

25 Appropriate amounts and dosages of that drug for inducing and/or maintaining anesthesia are appreciated and can be readily determined by persons having ordinary skill in the relevant art(s). Generally, it is expected that between about 0.5 to 1.5 mg of propofol per kilogram of a patient's body weight should be administered over a time period of about 60 seconds to induce anesthesia in an adult, human patient. A dosage of between about 100 and 150 µg of propofol per kilogram of a patient's body weight is preferably administered to maintain

30 anesthesia in the same patient.

The compositions can be parenterally administered to any animal, in particular, humans. In one embodiment, administration of a propofol containing composition comprises delivering the composition to a patient as a sole anesthetic, for example, via a bolus injection. In another aspect, administration of a propofol containing composition comprises delivering 5 the composition to a patient for the induction of anesthesia and subsequently maintaining anesthesia with another anesthetic. Alternatively, administration of a propofol containing composition comprises delivering the composition to a patient for the induction and maintenance of longer-term anesthesia, for example, via continuous infusion. Further, the compositions can be delivered to a patient via intramuscular (i.e., IM) means, e.g., IM 10 injection of propofol for induction and/or maintenance of anesthesia as well as other adjunct, desirable properties of compositions of the instant invention as described herein.

The propofol compositions comprise active agents in addition to propofol or, alternatively, the propofol compositions are co-administered with compositions comprising additional active agents. For example, the propofol containing compositions comprise or are 15 co-administered with one or more local anesthetic agents to reduce or eliminate injection pain. If administered, local anesthetic agents preferably are administered in concentrations sufficient to reduce or eliminate injection pain. One of ordinary skill in the art can select and administer concentrations of local anesthetic agent(s) to achieve the desired effects without undue experimentation.

20 The propofol containing compositions can be administered to a patient using techniques commonly known in the art. For example, the compositions can be delivered intravenously to a patient via bolus injection or by infusion. Infusion of the propofol containing compositions can be made by directly infusing a composition or, alternatively, by addition of a propofol containing composition to an appropriate infusion solution such as 25 0.9% sodium chloride injection, 5% dextrose injection, or another compatible infusion solution.

In one embodiment, the compositions of the present invention are withdrawn, prior to 30 administration, in multiple doses from a single container such as, for example, a vial or bag. For example, a composition of the invention is resistant to microbial growth even after multiple entries, e.g., with a syringe, into a single vial containing said composition. The multiple doses can be individually, or discretely, withdrawn such as by syringe or

continuously withdrawn such as by continuous intravenous infusion. For example, doses of the present compositions are repeatedly withdrawn from a single vial over a course of treatment. Alternatively, a single dose may be withdrawn from a container over a course of treatment.

5 In one embodiment, the composition of the present invention allows use from a multi-use container. For example, a multi-use container would allow individual doses to be withdrawn from the same container at different timepoints or different days. Multi-use containers can be fashioned in a variety of structures or methods known in the art. Multi-use containers may be particularly useful for anesthesia of animals.

10 The quantity of propofol and method of delivery to a patient during administration can be varied, as determined appropriate, by the physician supervising the administration.

In addition to conventional uses of propofol, such as its use in anesthesia, the administration of compositions of the present invention are useful as an antioxidant by administering an effective amount of propofol to a patient in need thereof. If anesthesia is 15 not desired, a sub-anesthetic dose may be administered in many cases. The propofol compositions of the present invention can be used for the prevention or reduction or treatment of oxidative injuries such as ischemia-reperfusion injuries. The propofol compositions can be used to inhibit oxidative damage induced by either hydrophilic or lipophilic radicals. The propofol compositions can be used to protect red blood cells and 20 brain, liver, kidney, heart, lung and skeletal muscle organs, tissue and cells from oxidative stress and injury by pretreatment of an individual with an effective amount of propofol. The propofol compositions of the present invention are also useful to inhibit platelet aggregation by administering an amount of propofol effective to inhibit platelet aggregation. Both the enhancement of antioxidant capacity and antiplatelet effect of propofol, and particularly the 25 propofol compositions of the present invention, make them particularly useful in coronary artery bypass surgery. In this indication propofol may be used, for example, at anesthetizing doses (for e.g., sufentanil 0.3 microg x kg(-1), propofol 1-2.5 mg x kg(-1) bolus then 100 microg x kg(-1) min(-1) before, and 50 microg x kg(-1) x min(-1) during CPB, or sufentanil 0.3 microg x kg(-1), propofol 2-2.5 mg x kg(-1) bolus then 200 microg x kg(-1) x min(-1)).

30 Small-dose propofol sedation can also be used to attenuate the formation of reactive oxygen species, and thus oxidative stress and injury, in tourniquet-induced ischemia-

reperfusion injury in patients under spinal anesthesia. An example of this use would be patients undergoing elective total knee replacement under intrathecal anesthesia.

Neuroprotection can further be provided by the propofol compositions, for e.g., by limiting the side-effect of vincristine in cancer therapy; reducing neural damage by

5 attenuating lactate accumulation and oedema formation in focal or global cerebral ischaemia; and reducing oxygen-centered free radical brain injury associated with trauma and stroke.

The propofol compositions of this invention may also be used for sedation. For example, lower doses (e.g. compared to the dose necessary for anesthesia) of propofol can have a sedative effect on a patient. Patients are often sedated during emergency room

10 procedures or prior to surgery to calm the patient.

Methods for administration and assaying the propofol compositions of the invention are routine in the art. Examples of methods of and assays can be found in: Runzer et al. Anesth Analg 2002 Jan 94(1):89-93; Eur J Anaesthesiol 2000 Jan 17(1):18-22; De La Cruz JP et al., Br J Pharmacol 1999 Dec; 128(7):1538-1544; Ansley DM et al., Can J Anaesth 15 1999 Jul 46(7):641-648; Murphy PG, et al., Br J Anaesth 1996 Apr 76(4):536-543; Daskalopoulos R et al. Glia 2002 Aug 39(2):124-132; Cheng YJ et al. Anesth Analg 2002 Jun 94(6):1617-1620; Wilson JX et al. J Neurosurg Anesthesiol 2002 Jan 14(1):66-79; Ergun R et al. Neurosurg Rev 2002 Mar 25(1-2):95-98; Li CR et al. Cell Biol Toxicol 2002 18(1):63-70.

20 In one aspect, the invention is directed to a composition of propofol which has a beneficial effect upon hemolysis of blood cells. The compositions of this invention may provide lower red blood cell lysis compared to emulsion propofol compositions, including but not limited to Diprivan. The compositions of this invention may also provide lower red blood cell lysis than saline solution. In a further aspect of this invention, the compositions of 25 this invention may stabilize red blood cell membranes.

In one aspect, the instant invention is directed to a sterile aqueous pharmaceutical composition comprising 2,6-diisopropylphenol, and one or more excipients; wherein the propofol red blood cell-blood plasma partition coefficient ( $K_p$ ) is about 3, is about 4, is about 5, is about 6, is about 7, is about 8, is greater than 3, is greater than 4, is greater than 5, is 30 greater than 6, is greater than 7, is greater than 8, is greater than 9, or is greater than 10. Further, the instant invention is directed to a sterile aqueous pharmaceutical composition

comprising 2,6-diisopropylphenol, and one or more excipients; wherein the propofol red blood cell-blood plasma partition coefficient ( $K_p$ ) for the composition is at least about two times, is at least about 3 times, is at least about 4 times, or is at least about 5 times the  $K_p$  obtained upon administration of a conventional propofol emulsion (e.g., Diprivan<sup>®</sup> or PropoFlo<sup>TM</sup> or Rapinovet<sup>TM</sup>) under the same delivery conditions. Additionally, the present invention includes a method of delivering propofol to a subject in need of anesthesia, the method comprising administering to a human or veterinary patient the sterile aqueous pharmaceutical composition described above. Preferably, the composition comprises two or more excipients, such as two or more surface active agents (e.g., two or more surfactants).

5 Preferably, the composition is substantially free of triacylglycerols. The composition can be further substantially free of other glyceryl esters of medium or long chain fatty acids or phospholipids. In one embodiment, the propofol red blood cell-blood plasma partition coefficient,  $K_p$ , for the composition is at least about 3 times the  $K_p$  obtained upon administration of a conventional propofol emulsion. In other embodiments, the propofol red

10 blood cell-blood plasma partition coefficient,  $K_p$ , for the compositions of the instant invention is at least about 3, at least about 4, or at least about 5.

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In another aspect, the instant invention is directed to a method of manipulating the blood plasma-red blood cell partition coefficient resulting from administration or delivery of a drug, for example a medicament or a therapeutic, diagnostic, or prophylactic agent such as propofol, to a patient. The blood plasma-red blood cell partition coefficient can be decreased or increased over the blood plasma-red blood cell partition coefficient resulting from administration or delivery of a conventional drug composition to a patient. Alternatively, compositions are prepared using the methods of the present invention that produce higher or lower blood plasma-red blood cell partition coefficients than compositions prepared using other methods. For example, particular formulations of the present invention are likely to increase the blood plasma-red blood cell partition coefficient resulting from administration or delivery of the instant propofol compositions over the blood plasma-red blood cell partition coefficient resulting from administration or delivery of Diprivan<sup>®</sup> Injectable Emulsion. The blood plasma-red blood cell partition coefficient is, for example, 2 or 3 times higher than the

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The method comprises preparing a pharmaceutical composition that comprises a drug and one or more excipients and wherein the pharmaceutical composition has a concentration of lipid excipients that is lower than the lipid concentration of an alternative composition comprising one or more lipids and wherein the alternative composition produces a lower

5 blood plasma-red blood cell partition coefficient upon administration or delivery to a patient. In one embodiment, the drug is lipophilic (i.e., the drug has an affinity for, tends to combine with, or is capable of dissolving in lipids). In a preferred embodiment, the pharmaceutical composition comprises two or more excipients. Preferably, at least one excipient of the composition is a surface active agent such as, but not limited to, a surfactant. In a preferred

10 embodiment, compositions are prepared that are substantially free of triacylglycerols. In one embodiment, the compositions are substantially free of other glyceryl esters of medium or long chain fatty acids or phospholipids as described herein. In one embodiment, the pharmaceutical composition is substantially free of lipid excipients.

The method comprises manipulating the concentration of lipid excipients to affect the

15 partition of a drug between blood plasma and red blood cells. For example, the concentration of lipid excipients is reduced to increase the amount of drug that enters red blood cells thereby increasing the blood plasma-red blood cell partition coefficient.

Alternatively, the excipients and excipient concentrations of the instant invention can be manipulated to yield a composition that produces a blood plasma-red blood cell partition

20 coefficient that is similar to that achieved by conventional drug formulations such as Diprivan® Injectable Emulsion. The excipients and excipient concentrations also can be manipulated to yield a composition that produces a blood plasma-red blood cell partition coefficient that is lower than that achieved by conventional drug formulations.

Methods for determining the blood plasma-red blood cell partition coefficient for a

25 delivered drug are well known to those of ordinary skill in the art. Preferably, the propofol red blood cell-blood plasma partition coefficient for comparison purposes is obtained upon administration of a conventional propofol emulsion such as, for example, Diprivan® Injectable Emulsion. Diprivan® Injectable Emulsion is a widely available, commercially sold pharmaceutical product. The composition of Diprivan® Injectable Emulsion is also stated

30 herein. Preferably, the conventional propofol emulsion and the composition of the instant invention are delivered under the same conditions. One of ordinary skill in the art can select

appropriate experimental conditions and determine the propofol red blood cell-blood plasma partition coefficients ( $K_p$ ) without undue experimentation.

Practice of the present invention provides several distinct advantages over

5 conventional propofol compositions, in particular, emulsion formulations. In one aspect, the present invention relates to propofol containing compositions, and their administration to a patient in need of anesthesia, that do not contain triacylglycerols. In another aspect, the propofol containing compositions do not contain phospholipids. Such compositions eliminate the substrate for bacterial growth that those lipids can provide. In contrast, the oil-in-water  
10 emulsions of conventional propofol formulations contain lipids such as, for example, soybean oil and lecithin that are able to support the growth of microorganisms. Conventional propofol formulations, composed of lipids, glycerol, and large amounts of water in an isotonic environment with neutral to alkaline pH, provide a medium quite conducive to the growth of many microorganisms. As such, these oil-in-water emulsions require stringent  
15 handling, administration, and storage requirements. By reducing or substantially eliminating the presence of triacylglycerols and other microorganism supporting lipids and providing physical and chemical stability, the compositions of the present invention allow for more flexibility in handling, administration, and storage. Less restrictive handling and storage requirements allow for improved and expanded administration options, for example, in  
20 remote makeshift hospital settings. Further, the compositions of the present invention, with reduced or no lipid content, minimize, if not eliminate, the potential for contributing to or causing hyperlipidemia.

The aqueous propofol compositions of the invention provide some advantages over other aqueous formulations.

25 The aqueous propofol compositions of the invention minimize or even eliminate the requirements for antimicrobials, such as disodium edetate, or preservatives such as benzyl alcohol to retard the growth of microorganisms. In addition, these compositions allow for more flexibility and efficiency in administration and packaging. For example, the compositions of the present invention allow packaging to contain multiple doses in contrast  
30 to the single dose form of the current commercial propofol emulsions necessitated by sterility concerns. Advantageously, practice of the instant invention allows the withdrawal of multiple

doses from a single vial over a period of time. Practice of the present invention also advantageously allows the use of tubing and opened portions of the propofol compositions for longer periods of time, e.g., longer than the currently recommended 12 hours, than are currently possible using conventional propofol compositions such as Diprivan® Injectable

5 Emulsion.

## 7. EXAMPLES

The present invention is also described and demonstrated by way of the following examples. However, the use of these and other examples anywhere in the specification is 10 illustrative only and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to any particular preferred embodiments described here. Indeed, many modifications and variations of the invention may be apparent to those skilled in the art upon reading this specification, and such variations can be made without departing the invention in spirit or in scope. The invention is therefore 15 to be limited only by the terms of the appended claims along with the full scope of equivalents to which those claims are entitled.

### 7.1. Preparation of Aqueous Propofol Samples

Exemplary propofol compositions can be prepared as follows and in accordance with 20 the present invention. First, the excipients listed in Table I, below, are weighed out and pre-dissolved in de-ionized water at room temperature. The solution pH of each preparation is then adjusted using 2N sodium hydroxide to obtain a final pH of about 5.0. De-ionized water is added to bring the total volume of each preparation up to 100 milliliters. Lastly, 1000 mg 25 of propofol and 200 mg of citric acid is added to each preparation, and the preparations are stirred using a magnetic stirrer at room temperature until the propofol was fully dispersed. Benzyl alcohol can also be included in the preparations, at an amount 0.45% (w/v).

Table I, below, lists the contents of each exemplary preparation together with the final percent concentration in weight/volume (w/v) for each ingredient in 100 ml of water.

**TABLE I: EXEMPLARY PROPOFOL FORMULATIONS**

Formulation Identity:	Poloxamer 188	PEG-400	Propylene glycol	Propofol
M841	8 %	4 %	1 %	1 %
M831	8 %	3 %	1 %	1 %
M840	8 %	4 %	0 %	1 %
M830	8 %	3 %	0 %	1 %
M741	7 %	4 %	1 %	1 %
M740	7 %	4 %	0 %	1 %
M731	7 %	3 %	1 %	1 %
M730	7 %	3 %	0 %	1 %
M641	6 %	4 %	1 %	1 %
M642	6 %	4 %	2 %	1 %
M661	6 %	6 %	1 %	1 %
M660	6 %	6 %	0 %	1 %
M920	9 %	2 %	0 %	1 %

## 7.2. Physical Properties of Aqueous Propofol Preparations

The physical properties of aqueous propofol preparations, such as those described in  
5 the above example, can be evaluated as follows. First, the preparations' appearance (clear,  
hazy or less hazy) is evaluated with the naked eye to determine whether visible solids are  
present.

Particle sizes can also be estimated using Laser Light Scattering (LLS) particle size  
analysis, *e.g.*, with a Zetasizer 300 HS available from Malvern Instruments, Inc.  
10 (Southborough, Massachusetts) according to the manufacturer's instructions. The  
polydispersity index can then be calculated for a formulation using the LLS data. Osmolality  
of a preparation can be determined, *e.g.*, using a Vapor Pressure Osmometer (VAPRO),  
available from WESCOR, Inc. (Logan, Utah).

The physical stability of different preparations can be initially estimated by storing  
15 the preparations at room temperature and measuring one or more of the above-listed

properties at periodic intervals (preferably at least once each day) to determine if an/or when the number or size of particles within the preparations changes.

Exemplary results for the formulations described in the previous example are set forth  
5 in Table II, below.

**TABLE II: PHYSICAL PROPERTIES OF  
EXEMPLARY PROPOFOL FORMULATIONS**

<b>Formulation Identity:</b>	Avg. Particle Size (nm)	Polydispersity Index	Osmolality (mmol/kg)	Appearance	Physical Stability* (days)
M841	56	0.21	299	Clear	14
M831	55	0.22	292	Clear	14
M840	50	0.23	175	Clear	14
M830	57	0.21	162	Clear	14
M741	51	0.19	302	Clear	14
M740	47	0.17	295	Clear	14
M731	50	0.19	255	-	-
M730	49	0.18	153	-	-
M641	-	-	-	Hazy	X†
M642	50	0.15	400	Hazy	X†
M661	54	0.16	370	Less Hazy	X†
M660	-	-	-	Less Hazy	X†
M920	50	0.21		Clear	13

\* Time during which no change in particle size is detected.

† “X” indicates that the sample is not a clear solution after fourteen days, and is not considered physically stable.

Data from a more detailed analysis of two preferred formulations (M841 and M831) are shown below, in Table III. Briefly, preparations containing 2 mg/ml citric acid and

10 0.45% benzyl alcohol or, alternatively, 2 mg/ml and no benzyl alcohol are stored at 80 °C and at 4 °C. The initial particle size and polydispersity index is measured as described above for each sample immediately before storage and at periodic intervals thereafter. The appearance of each preparation to the naked eye is also evaluated to determine whether it is clear or cloudy, indicating that propofol particles may have separated from the solution.

15 The different propofol formulations are stable for as long as four weeks across these various temperatures, and the presence or absence of benzyl alcohol does not adversely affect the physical stability of these preparations. The preparations remain as clear solutions at

storage temperatures between about 25 and 80 °C. However, at temperatures below about 25 °C the preparations become cloudy and the phase separates. The temperature at which this phase separation occurs is therefore referred to as the “cloud point,” and can be determined by evaluating the physical appearance of preparations (clear or hazy) as they are slowly cooled. For example, the cloud point of the formulation M831 (with 2 mg/ml citric acid and 0.45% benzyl alcohol) is determined to be about 13 °C, whereas the cloud point of M831 (2 mg/ml citric acid) without benzyl alcohol is very similar – about 16 °C. The data shown in Table III, below, show that even after storage at a temperature below the cloud point, formulations of the invention can be reconverted to a clear solution by allowing them to equilibrate at a temperature above the cloud point.

**TABLE III: PHYSICAL STABILITY OF PROPOFOL FORMULATIONS AT DIFFERENT STORAGE TEMPERATURES**

<b>Formulation:</b>	<b>Initial Value</b>	<b>Stored at 80 °C</b>		<b>Stored at 80 °C</b>	
		<b>2 weeks</b>	<b>4 weeks</b>	<b>2 weeks*</b>	<b>2 weeks†</b>
M841 (w/ benzyl alkohol)	47 nm (0.25)	38 nm (0.18)	38 nm (0.17)	46 nm (0.19)	42 nm (0.17)
M841 (w/o benzyl alcohol)	55 nm (0.21)			46 nm (0.19)	42 nm (0.17)
M831 (w/ benzyl alcohol)	47 nm (0.25)	36 nm (0.17)	37 nm (0.17)	48 nm (0.22)	41 nm (0.17)
M831 (w/o benzyl alcohol)	55 nm (0.21)	42 nm (0.16)	42 nm (0.16)	97 nm (0.70)	54 nm (0.16)

\* Measured after equilibrating at room temperature for three hours.

† Measured after equilibrating at room temperature for two days.

### 7.3. Chemical Stability of Aqueous Propofol Preparations

The chemical stability of aqueous propofol preparations, such as those described in the above examples, can be evaluated using routine techniques. For example, propofol containing compositions can be subjected to a variety of environmental conditions, after which the stability of propofol and/or the excipients is evaluated using high performance liquid chromatography (HPLC). Briefly, the sample can be diluted in an aqueous mixture containing 60% acetonitrile, and injected onto a C18 column operated at 45 °C. A two component gradient mobile phase is run consisting of: (a) 0.05% TFA in water, and (b) 0.05% in acetonitrile. The run time is preferably about 55 minutes with a flow rate of

0.8 ml/min. Propofol will typically elute from the C18 column after about 21 minutes. Eluting propofol can be detected, along with various impurities and degradation products, by monitoring the elute's UV absorption at 272 nm. The amounts of different impurities can be estimated by measuring the area under their peak's on the HPLC chromatogram, and

5 normalizing that area to the area under the peak corresponding to propofol.

**Figures 1 - 4** each show the exemplary results of such an analysis for various propofol preparations stored over time (indicated in weeks) under different environmental conditions. In each instance, the amount of propofol remaining in each preparation (indicated as percentage of the initial propofol content) can be estimated from the peak area

10 of propofol, which elutes from the C18 column about 21 minutes into the gradient run.

In more detail, **Figure 1** shows exemplary results for various propofol formulations from Table I, *supra*, that are stored at 80 °C, to simulate non-ideal or "stressed" storage conditions to which a propofol formulation may be subjected. These data indicate that all of the compositions are highly stable under such conditions, with less than 0.2% propofol

15 degradation even after four weeks of storage under these conditions. Because formulations with minimal levels of excipients are generally preferred, the formulation M831 was selected for further analysis.

**Figures 2A-2B** show exemplary data from the analysis of the M831 formulation stored at 80 °C at a under a variety of different pH conditions, demonstrating that the

20 formulation is remarkably stable at pH levels as high as about 6.2. **Figure 3** shows exemplary data from the analysis of M831 formulations (stored at pH 5 and 80 °C) containing different levels of citric acid. These data demonstrate that the presence of citric acid in aqueous propofol formulations of the invention (preferably at concentrations of about 2 mg/ml) greatly improves the stability.

25 **Figure 4** shows exemplary data from the HPLC analysis formulations M831 and M830, which contain 1% and 0% propylene glycol (w/v), respectively. Both samples additionally contain benzyl alcohol (0.45% w/v) and citric acid (2 mg/ml). In these data, the purity of each preparation is estimated by measuring the area under curves associated with degradation production of propofol. These data show that the amount degradation products

30 increases rapidly in formulations containing no propylene glycol, whereas the level of

degradation products remain relatively stable in degradation products that contain a small amount (*e.g.*, about 1%) or propylene glycol.

#### **7.4. Commercial Manufacture of Aqueous Propofol Formulations**

5       Aqueous propofol formulations of the present invention can be readily manufactured in a manner substantially similar to the preparation of laboratory samples described in the preceding examples. As an example and without being limited to any particular method or embodiment, purified water for injection (WFI) and block polymer (*e.g.*, poloxamer 188) can be combined in a formulation vessel and stirred until the block polymer is dissolved. Other  
10      excipients (preferably a polyethylene glycol such as PEG-400, a propylene glycol, citric acid and benzyl alcohol) are then added and mixed until all of these ingredients are dissolved. The pH is then adjusted to  $5.5 \pm 0.5$  using sodium hydroxide solution. Propofol is then added and the solution is mixed until propofol is fully dissolved.

15      Water for injection is then added to achieve a target batch weight. The final formulation is then sterilized by filtration through a 0.2  $\mu\text{m}$  nominal pore sized filtered. The filtered solution can be aseptically filled into glass vials, stoppered and capped. Preferably, the finished vials are inspected for particles or other defects before packaging.

20      In the above described method, block polymer is preferably added first, followed in order by pH titration and the addition of propofol. The order in which other ingredients are added to the composition is not significant.

#### **8. REFERENCES CITED**

25      Various references, including patents, patent applications and various publications, are cited and discussed in the description of this invention. The citation and/or discussion of any such reference is provided merely to clarify the description of the present invention and is not an admission that any such reference is “prior art” to the invention described here. All references cited and/or discussed in this specification are incorporated herein by reference in their entirety and to the same extent as if each reference was individually incorporated by reference.